CDK/GSK-3 inhibitors as therapeutic agents for parenchymal renal diseases

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Drug discovery to lessen the burden of chronic renal failure and end-stage renal disease remains a principle goal of translational research in nephrology. In this review, we provide an overview of the current development of small molecule cyclin-dependent kinase (CDK)/glycogen synthase kinase-3 (GSK-3) inhibitors as therapeutic agents for parenchymal renal diseases. The emergence of this drug family has resulted from the recognition that CDKs and GSK-3s play critical roles in the progression and regression of many kidney diseases. CDK/GSK-3 inhibitors suppress pathogenic proliferation, apoptosis, and inflammation, and promote regeneration of injured tissue. Preclinical efficacy has now been demonstrated in mesangial proliferative glomerulonephritis, crescentic glomerulonephritis, collapsing glomerulopathy, proliferative lupus nephritis, polycystic kidney diseases, diabetic nephropathy, and several forms of acute kidney injury. Novel biomarkers of therapy are aiding the process of drug development. This review will highlight these advancements in renal therapeutics.

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Many drugs used in the renal clinic today originated from their empiric administration to patients with parenchymal renal diseases, despite limited knowledge of disease pathogenesis or molecular drug targets. Seminal clinical observations several decades ago of therapeutic responses from nitrogen mustard,[1] steroids,[2] and cyclophosphamide[3] in various nephritic and nephrotic renal lesions laid the early foundation for the ongoing empiric use of immunomodulatory drugs to treat many kidney diseases. Recent 'top-down' translational research[4] to better understand how these drugs may confer efficacy, however, continue to uncover unexpected mechanisms of drug action,[5,6] underscoring recurring themes in drug discovery: efficacy often results from the integrative rather than the singular action of drugs, and the specificity of drug action is contextual and differs depending on how the molecular targets of drugs promote or resolve any particular disease.

Here, we discuss the development of small molecule cyclin-dependent kinase (CDK)/glycogen synthase kinase-3 (GSK-3) inhibitors (CGIs) as therapeutic agents for parenchymal renal diseases. In contrast to discoveries through 'top-down' translational research, the growing interest in CGIs has come via the 'bottom-up' recognition[4] in molecular nephrology that CDKs and GSK-3s are integrally involved in the pathogenesis and repair of many forms of renal injury. Because these paralogous kinases enter into several biologic processes both within and outside the kidney,[7–14] CGIs share with many other renal therapeutic agents the propensity for promiscuity in achieving efficacy.[15,16] This is also evidenced by the ongoing development of CGIs for use in many other diseases, including cancer, diabetes, cardiovascular and neurodegenerative disorders, several viral and parasitic infections, systemic inflammatory syndromes, and in regenerative medicine.[7–14]

CGIs AND KINASE SELECTIVITY

The CDK and GSK-3 families of serine/threonine kinases are closely related phylogenetically,[17,18] and studies in chemical proteomics[19,20] uncovered their overlapping sensitivity to several chemically diverse small molecules.[21–25] Twenty CDKs (CDK-1 through CDK-13 plus seven CDK-like kinases for which cyclin-binding partners have not been identified) and

two GSK-3s (GSK-3α and GSK-3β) exist in the mammalian kinome and share high structural similarity at their ATP-binding and catalytic domains.[17,18,25] Despite the divergent feature that CDKs but not GSK-3s typically require protein-binding partners (that is, cyclins) to induce kinase activity,[7,8] both families are inhibited by several of the same CGIs.[21–25] In most cases, this occurs because these 'pan-selective' CGIs compete with ATP for docking at the ATP-binding pocket within CDKs and GSK-3s at similar potencies.[21–25] These pan-selective CGIs constitute a diverse repertoire of small molecules and include analogues of the purines, pyrimidines, maleimides, flavones, indirubins, along with members of many other chemical classes.[21–25]

Modifications around the chemical scaffold of many pan-selective GCIs can logarithmically increase or decrease selectivity for specific CDK and GSK-3 family members.[21–25] This selectivity is typically demonstrated by *in vitro* assays utilizing purified kinases, representative substrates, and steady-state concentrations of drug.[21–25] Whether this selectivity exists during drug administration *in vivo*, however, is less clear. As with all other small molecule drugs, the pharmacokinetics and pharmacodynamics of each CGI factors importantly into its target selectivity during treatment regimens.[26] Indeed, in the absence of type I biomarkers to directly detect and monitor the modulation of potential drug targets *in vivo*, the selectivity of CGIs *in vivo* is largely extrapolated from *in vitro* kinase assays, cell culture-based assays, and surrogate/type II biomarkers of drug activity.[15,27] Combined with the fact that CGIs (along with other kinase inhibitors) have not been screened against all known kinases or other nucleotide-interacting proteins, delineating the selectivity of CGIs remains an important focus of research on this drug family.[19,20] Table 1 lists the CGIs that have been studied in renal diseases to date along with their *in vitro* kinase inhibitory profiles.

PATHOGENIC AND REPARATIVE PHENOTYPES TARGETED BY CGIs

CDKs and GSK-3s are involved in several essential physiologic responses,[7–14] and their aberrant activities have been implicated in the pathogenesis of multiple parenchymal renal diseases. Figure 1 depicts several of the major molecular pathways controlled by CDKs and GSK-3s, which have been modulated by GCIs to preserve renal function. Of disease phenotypes, the ability of GCIs to suppress proliferation, apoptosis, and inflammation has received the greatest attention. Because these phenotypes often coexist and synergize to promote progressive injury to the renal parenchyma, CGIs harbor the inherent ability to simultaneously target multiple aspects of disease pathogenesis. Table 2 lists the therapeutic responses to CGIs by various cell types both within and outside the kidney that have been implicated in the development of parenchymal renal diseases.

CGIs may also promote remodeling and repair of injured renal parenchyma (Figure 1). The GSK-3β inhibitor, lithium, and the CGI, 6-bromoindirubin-3′-oxime (BIO), promote survival of growth factor-deprived renal epithelial cells by activating the Wnt pathway.[57] Wnt signaling is induced in renal epithelial cells subjected to ATP depletion–repletion, causing nuclear translocation of β-catenin to activate lymphocyte enhancer-binding factor/T cell factor (LEF/TCF)-mediated gene expression.[58] In response to ischemia–reperfusion injury, Wnt-4 expression increases, and, via β-catenin, upregulates the expression of cyclin D1 to promote cell-cycle progression.[59] Thus, activation of Wnt signaling in response to renal injury may promote the repair of injured renal parenchyma, preserving renal function.

Wnt signaling via GSK-3β is also a key regulator of the pluripotency and function of stem cells,[14] a focus of increasing importance in regenerative medicine of the kidney.[60] Current evidence suggests that bone marrow stem cells facilitate the recovery of injured renal parenchyma by differentiating into renal parenchymal cells and/or by acting in a paracrine manner to facilitate the local intrarenal regenerative response.[61–66] Additionally, renal stem cell niches reside within the kidney,[67,68] from which progenitors may be recruited locally to facilitate repair of injured parenchyma.[60] Thus, CGIs may have therapeutic applications in regenerative therapies for the kidney.[14] Proof of this concept already exists for some CGIs that have been shown to sustain the pluripotent state of embryonic stem cells *in vitro*,[69] to promote hematopoietic stem cell repopulation *in vivo*,[70] and to promote nephrogenesis *ex vivo*.[56]

Table 1 | Kinase inhibitory profiles (IC_{50} in μM) for CGIs studied in renal diseases

Inhibitor[a]	Class	CDK-1	CDK-2	CDK-4	CDK-5	CDK-7	CDK-9	GSK-3α/β	Refs.
6-Bromo-indirubin-3′-oxime	Indole	0.32	0.3	10	0.08	—	—	0.005	Meijer *et al.*,[23] Polychronopoulos *et al.*[28]
Flavopiridol	Flavone	0.06	0.15	0.4	0.17	0.3	0.006	0.45	Leclerc *et al.*,[22] Chao *et al.*[29]
Olomoucine	Purine	7	7	>100	3	—	—	100	Vesely *et al.*[30]
Purvalanol B	Purine	0.006	0.006	>10	0.006	—	—	>10	Leclerc *et al.*,[22] Gray *et al.*[31]
R-roscovitine	Purine	0.45	0.13	14.7	0.16	0.49	0.78	130	Leclerc *et al.*,[22] Raynaud *et al.*[32]
SB216763	Maleimide	0.55	—	—	—	—	—	0.034	Meijer *et al.*,[11] Coghlan *et al.*[33]
SB415286	Maleimide	0.9	—	—	—	—	—	0.078	Meijer *et al.*,[11] Coghlan *et al.*[33]
TDZD-8	Thiadiazolidinone	>10	—	—	—	—	—	2	Meijer *et al.*,[11] Martinez *et al.*[34]

CDK, cyclin-dependent kinase; CGI, cyclin-dependent kinase/glycogen synthase kinase-3 inhibitor; IC_{50}, half-maximal inhibitory concentration; TDZD-8, thiadiazolidinone-8.
[a]The full profile of some CGIs is not known.

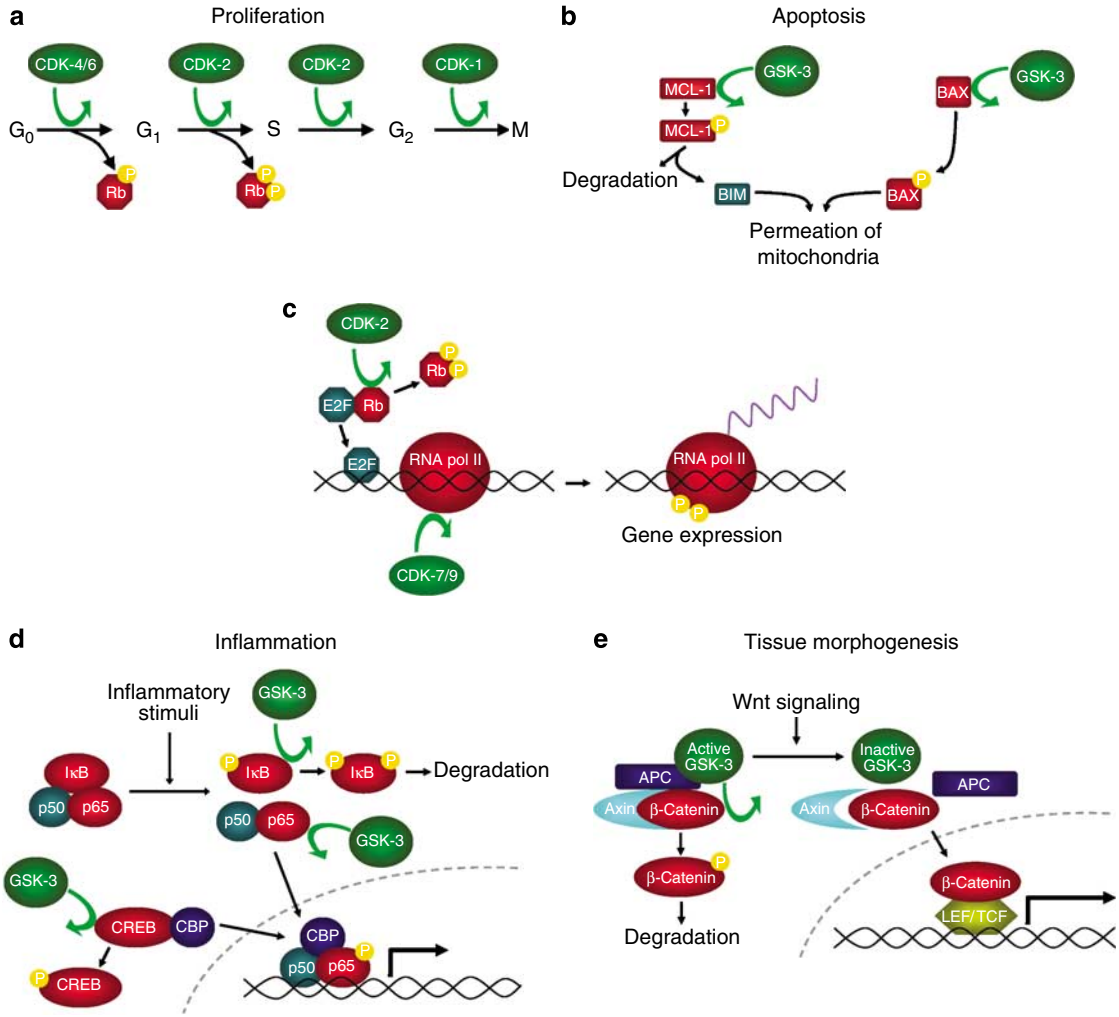

Figure 1 | Molecular pathways of proliferation, apoptosis, inflammation, and tissue morphogenesis that have been modulated by CGIs to preserve renal function. The kinase targets of CGIs and their substrates are shown in green and red, respectively. (**a**) CDKs promote engagement (G_0 to G_1) and progression (G_1 to M) of the cell cycle. Among several substrates, phosphorylation (P) of the retinoblastoma protein (Rb) by CDKs allows for proliferating cells to transition into the S phase of the cell cycle. (**b**) GSK-3s can phosphorylate both MCL-1, increasing free BIM, and BAX. Both events promote permeation of mitochondria, thereby releasing cytochrome *c* and activating proapoptotic cascades in cells. (**c**) CDKs can also influence proliferation, apoptosis, and inflammation through direct control of gene expression. One example is the liberation of E2F transcription factors and expression of E2F-dependent pro- or antiapoptotic genes following phosphorylation of Rb by CDK-2. Another example is the phosphorylation of RNA polymerase (pol) II by CDK-7 and CDK-9, thereby supporting expression of short-lived mRNAs required for cell-cycle progression. (**d**) GSK-3s promote proinflammatory NF-κB-mediated gene expression. Multiple steps in the activation of NF-κB transcription factors have been implicated, including phosphorylation of IκB and the p65 subunit of NF-κB, and release of the NF-κB transcription cofactor, CBP, from sequestration by CREB following phosphorylation of CREB. (**e**) GSK-3s are central to tissue morphogenesis and repair in the kidney, as exemplified by the role of GSK-3s in determining the fate of β-catenin in Wnt signaling. IκB, inhibitor κB; MCL-1, myeloid cell leukemia-1. CREB, cAMP response element-binding protein; CBP, CREB-binding protein.

PRECLINICAL EFFICACY IN MANY KIDNEY DISEASES

Mesangial proliferative glomerulonephritis

The first preclinical study providing proof-of-concept that CGIs may be efficacious in proliferative and immunologic renal diseases utilized the rat anti-Thy1.1 model of mesangial proliferative glomerulonephritis.[35] Administration of roscovitine to rats either before or 3 days after induction of mesangial proliferative glomerulonephritis with anti-Thy1.1 antibody markedly decreased mesangial cell proliferation, mesangial matrix production, and renal insufficiency as measured by urine (protein/creatinine) and blood urea nitrogen levels at day 10 after disease onset. This groundbreaking report contributed to the rationale for clinical trials of roscovitine in patients with IgA nephropathy.[15]

Crescentic glomerulonephritis and collapsing glomerulopathy

The diagnoses of crescentic glomerulonephritis and collapsing glomerulopathy, nephritic and nephrotic manifestations of glomerular epithelial cell proliferation, respectively,

Table 2 | Therapeutic responses by various cell types implicated in the progression and regression of renal diseases

Therapeutic response	Proposed specificity of action of CGIs	Refs.
Antiproliferative		
Mesangial cell	Cell-cycle arrest by inhibiting cell cycle and transcriptional CDKs	Pippin *et al.*,[35] Zoja *et al.*[36]
Podocyte	Cell-cycle arrest by inhibiting cell cycle and transcriptional CDKs	Griffin *et al.*,[37] Nelson *et al.*,[38] Nelson *et al.*,[39] Nelson *et al.*,[40] Gherardi *et al.*[41]
Renal tubular epithelium	Cell-cycle arrest by inhibiting cell cycle and transcriptional CDKs	Bukanov *et al.*[42]
Antiapoptotic		
Mesangial cell	Lack of caspase activation by inhibiting CDK-2; stabilization of β-catenin by inhibiting GSK-3β	Lin *et al.*,[43] Hiromura *et al.*[44]
Renal tubular epithelium	Lack of caspase activation by inhibiting CDK-2 or CDK-5; lack of E2F1 activation by inhibiting CDK-2	Bukanov *et al.*,[42] Price *et al.*,[45] Price *et al.*,[46] Price *et al.*,[47] Yu *et al.*[48]
Anti-inflammatory		
Renal tubular epithelium	Suppression of NF-κB-mediated expression of cytokines and chemokines by inhibiting GSK-3β; suppression of expression of pattern recognition receptors	Dugo *et al.*,[13] Benigni *et al.*,[49] Gong *et al.*[50]
Renal endothelium	Suppression of NF-κB-mediated expression of adhesion molecules by inhibiting GSK-3β	Gong *et al.*,[51] Gong *et al.*[52]
T lymphocytes	Suppression of proliferation and cytokine secretion by inhibiting CDKs	Zoja *et al.*[36]
B lymphocytes	Suppression of proliferation and immunoglobulin production by inhibiting CDKs	Zoja *et al.*[36]
Macrophages and myeloid dendritic cells	Suppression of differentiation and proinflammatory activation by inhibiting GSK-3β	Martin *et al.*,[53] Rodionova *et al.*[54]
Neutrophils	Debulking via enhanced apoptosis by inhibiting CDKs	Rossi *et al.*[55]
Reparative		
Renal tubular epithelium	Cell-cycle engagement via activation of Wnt signaling by inhibiting GSK-3β	Kuure *et al.*[56]
Renal stem cell niches	Maintenance of pluripotency and recruitment by inhibiting GSK-3β	Romagnani *et al.*,[14] Sinha *et al.*[57]

CDK, cyclin-dependent kinase; CGI, cyclin-dependent kinase/glycogen synthase kinase-3 inhibitor; GSK-3β, glycogen synthase kinase-3β; NF-κB, nuclear factor-κB.

portend rapid losses in renal function. Administration of roscovitine to rats with the nephrotoxic form of crescentic glomerulonephritis or to mice with the anti-total glomerular protein form of crescentic glomerulonephritis/collapsing glomerulopathy ameliorated crescent formation, glomerular matrix deposition, and the accompanying renal insufficiency.[16,37] Treatment of Tg26 mice, an established model of HIV-induced collapsing glomerulopathy, with flavopiridol or roscovitine prevented and reversed existing renal disease.[38–41] Because CGIs could also suppress HIV gene expression, these studies in Tg26 mice demonstrated for the first time that CGIs may simultaneously target both the etiologic and phenotypic manifestations of renal disease.[38–41] The safety of CGIs in states of glomerular epithelial cell injury was also supported by studies in the passive Heymann nephritis model of membranous nephropathy.[71]

Proliferative lupus nephritis

The ability of CGIs to target both intrarenal and extrarenal contributions to parenchymal injury was recently demonstrated in the (NZB × NZW) mouse model of proliferative lupus nephritis.[36,49] Administration of roscovitine to (NZB × NZW) mice with early or established proliferative lupus nephritis significantly reduced glomerular hypercellularity, tubulointerstitial damage, leukocyte inflammation, immunoglobulin and complement deposition, and proteinuria. When roscovitine was combined with low-dose steroids, the survival of (NZB × NZW) mice was markedly enhanced when compared to either agent alone. These studies demonstrated that direct suppression by roscovitine of the extrarenal, autoimmune T- and B-lymphocyte responses contributed to the amelioration of proliferative lupus nephritis.

Polycystic kidney diseases

Polycystic kidney diseases (PKDs), the leading genetic disorders for end-stage renal disease, are caused by dysfunctional cilia that trigger abnormal proliferation, apoptosis, and dedifferentiation of tubular epithelium.[72] Treatment of *jck* and *cpk* mice, models of slowly and rapidly progressive forms of PKDs, respectively, with roscovitine induced long-lasting arrest of renal cystogenesis along all segments of the nephron.[42] This occurred even with interrupted dosing regimens, suggesting sustained correction of the abnormal renal epithelial phenotype with drug treatment. Efficacy correlated with roscovitine's ability to induce cell-cycle arrest, suppress apoptosis, and attenuate the heightened activity of RNA polymerase II in diseased kidneys.

Diabetic nephropathy

High-glucose-induced apoptosis of mesangial cells contributes to the development of glomerulosclerosis in diabetic nephropathy, a sequelae of signaling by GSK-3β in mesangial cells.[43] Administration of BIO to rats with streptozotocin-induced diabetic nephropathy suppressed mesangial cell apoptosis, as measured by TUNEL (TdT-mediated dNTP nick end labeling) staining *in situ*, and ameliorated proteinuria.[43] Since GCIs are also being developed to potentiate insulin signaling as a strategy to lower glucose levels in type II diabetes,[12] their use in diabetic nephropathy represents another kidney disease in which GCIs may target both the etiologic and phenotypic manifestations of renal injury.

Acute kidney injury

In acute kidney injury (AKI), damaged renal epithelial cells either recover to promote repair or undergo cell death. The endogenous CDK-2 inhibitor, p21, has been suggested from *in vitro* and *in vivo* models of ischemic and cisplatin-induced AKI to serve as the cell-cycle checkpoint governing this response.[73,45–48] Induction of p21 during AKI allows injured cells to undergo repair prior to cell replication, whereas in the absence of p21, injured cells prematurely engage E2F1-dependent cell-cycle and apoptotic pathways, promoting further injury.[73,45–48] Indeed, roscovitine and purvalanol were found to protect from cisplatin-induced AKI with preservation of kidney morphology and function.[46,47]

CGIs also attenuate AKI in the setting of systemic inflammatory response syndromes.[13] A central pathogenic feature of systemic inflammatory response syndromes is the robust and systemic release of multiple NF-κB-dependent proinflammatory mediators that have detrimental effects on several organs, including the kidney.[13] Because of the direct control of NF-κB activation by GSK-3β, TDZD-8, SB216763, and SB415286 were tested in several preclinical models of systemic inflammatory response syndromes and found to markedly suppress proinflammatory cytokine release and ameliorate AKI.[13] It is unknown but reasonable to consider that these CGIs also modulated NF-κB-independent pathways within the renal parenchyma that also contributed to their efficacy in these studies (Figure 1).

THE CHALLENGE OF CLINICAL DEVELOPMENT

The high rates of attrition of promising therapeutic agents such as CGIs during preclinical-to-clinical development is a well-recognized challenge of new drug discovery.[15,74,75] While the reasons for this are multifactorial, leading among them are problems with absorption, distribution, metabolism, excretion, and toxicity (ADMET).[26,76] This exists not only for new drug entities but also in the off-label use of Food and Drug Administration (FDA)-approved drugs for renal diseases where ADMET is poorly understood.[77] For example, clinical trials of the cancer drug, Gleevac, in IgA nephropathy were halted over concerns of potential toxicities with its prolonged use in this typically indolent disease.[15] Notwithstanding, attrition remains a force for exploring alternative therapeutic activities of established drugs in parenchymal renal diseases, as exemplified, among many others, by the retinoids,[78] 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors,[79] and peroxisome proliferator-activated receptor-γ agonists.[80]

Another common reason for attrition is that preclinical efficacy may not predict clinical efficacy.[81] This is particularly true when preclinical studies do not mimic the clinical reality of treating existing renal disease, often the case with patients in nephrology. Most of the preclinical studies listed above tested treatment, not just prevention, by CGIs. (One notable exception is prophylaxis for AKI, a relatively minor clinical need compared to the burden from established AKI.) Yet, no animal model of renal disease can exactly recapitulate the varieties of patients. Moreover, due to a paucity of type I biomarkers, most preclinical studies utilize type II biomarkers to infer the specificity of drug action, which may ultimately differ in clinical settings.[15,27] Indeed, many existing type II biomarkers such as creatinine, while uninformative of drug mechanism, may also not be sensitive enough to detect early responses to CGIs to help in selecting lead drug candidates (discussed next).

BIOMARKERS OF THERAPY

The translation of CGIs into renal therapeutic agents could be accelerated by the discovery and implementation of novel biomarkers of therapy.[15,27,82,83] Many parenchymal renal diseases that may benefit from CGIs are not only difficult to diagnose early but also progress with significant variability. As a result, the task of delineating the specificity and efficacy of CGIs with existing biomarkers may become quite complex and cumbersome. For example, the Consortium for Radiologic Imaging Studies of PKD cohort recently determined that quantitative magnetic resonance imaging could predict the clinical progression and, presumably, regression of PKD in patients with kidney volumes greater than 1500 ml.[84] However, this sophisticated imaging modality as a biomarker to determine responses to therapy is not widely available and may not capture the efficacy of CGIs in PKD patients with smaller kidneys.[85]

Studies of the urine proteome in PKD exemplifies how novel biomarkers may facilitate the development of CGIs.[82,83] Application of SELDI-TOF (surface-enhanced laser desorption/ionization time-of-flight) mass spectrometry[86] to fractionate proteins in the urine of wild-type mice and *jck* mice with PKD creates a banding pattern by gel view that readily discriminates between normal and diseased kidneys (Figure 2; N Bukanov, personal communication). During administration of roscovitine to ameliorate PKD,[42] the SELDI-TOF banding pattern of the urine proteins from *jck* mice returns to one that is similar to that of wild-type mice, indicating establishment of a more normal phenotype and physiologic loss of proteins from diseased tubular epithelium with therapy. Thus, assaying for differentially excreted urine proteins in experimental animals and humans during therapy

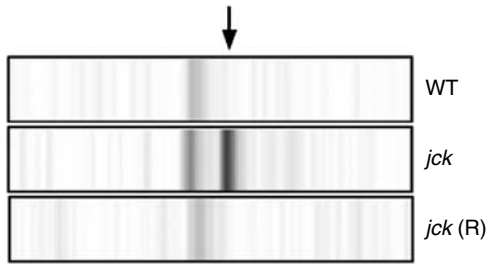

Figure 2 | The urine proteome as a biomarker of therapy with CGIs. SELDI-TOF mass spectroscopy was used to fractionate and profile differences between proteins present in the urine of wild-type mice (WT), PKD mice (*jck*), and PKD mice treated with roscovitine (*jck* (R)). These representative gel views demonstrate a prominent band (indicated with an arrow) that exists in the urine of mice with PKD, which is absent in the urine of wild-type mice or in the urine of mice with PKD treated with roscovitine. The overall banding pattern of proteins in the urine of wild-type mice and roscovitine-treated mice is similar.

could provide a valuable and rapid tool to screen and validate GCIs, and ultimately guide drug development and therapy.[82,83] Although these types of biomarkers of therapy clearly require further study, they may be extremely useful in the development of CGIs as renal therapeutic agents.

CONCLUDING REMARKS

GCIs are promising new therapeutic agents for many parenchymal renal diseases. Cumulative evidence suggests that CGIs confer efficacy through multiple mechanisms that may include the targeting of intrarenal and extrarenal components of disease pathogenesis and repair, and the etiology for renal injury. Depending on the context in which CDKs and GSK-3s enter into the progression or regression of specific parenchymal renal diseases, the selectivity of CGIs and the timing of drug treatment for optimal therapeutic benefit are important areas of future research on this drug family. This inquiry will be aided by the application of novel biomarkers of drug mechanism and therapy that should accelerate the drug discovery process and the use of CGIs in the renal clinic.

DISCLOSURE

Oxana Ibraghimov-Beskrovnaya and Anna Zuk are employees of the Genzyme Corporation. Laurent Meijer is a founding member of ManRos Therapeutics.

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